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TITLE: Molecular Epidemiology of Ovarian Cancer

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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

The aim of this Program is to study the association between epidemiologic risk factors, low-risk genes, and histologic and novel molecular subtypes of ovarian cancer. Funding for the study began in September 2001 and we have since established systems for case ascertainment and recruitment at each of the collaborating sites. Data-collection instruments have been finalised and piloted (using local pilot funding) at the Royal Women's Hospital, Brisbane and at the Mater and Wesley Hospitals and 90% of eligible cases (82 patients) have consented to participate and completed the questionnaires. Project managers for the epidemiology and biospecimen cores have been appointed. A tracking database has been established and the biospecimen database substantially upgraded. SNP detection methodologies have been established. Our major task has been obtain IRC approval at 14 participating sites (complete except for one site), and subsequently obtaining HSRRB approval. Given the Australian context, some of the IRC's have not had an USA FWA in place. This has necessitated obtaining SPA at such sites. All centres in South Australia and NSW have both FWA and IRC approval, and we believe that the study will commence very soon in those states, pending final HSRRB approval.

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TABLE OF CONTENTS

ANNUAL REPORT

Title: Molecular Epidemiology of Ovarian Cancer

Proposal No. OC000109
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INTRODUCTION

The aim of this Program is to study the association between epidemiologic risk factors, low-risk genes, and histologic and novel molecular subtypes of ovarian cancer, thereby addressing the heterogeneity of the disease and of susceptibility to environmental exposures. To this end, we will establish a multi-center population-based resource involving collection of epidemiologic data and biospecimens, including fresh tumor tissue, from cases and matched controls.

BODY

We have been involved in a lengthy process of meeting requirement of Institutional Review Board (IRB) at participating sites around Australia and subsequently gaining approval from the Contracting Officer. IRB approval is complete at all but one participating site.

The Human Subject Research Review Board (HSRRB) has been reviewing our documents (research protocols, consent forms) to determine whether they comply with all the human subject protection regulations. This review procedure began in November 2001 and Andrea Kline (Human Subjects Protection Specialist, AMDEX Corporation) has been instrumental in facilitating this process. We received a letter from Andrea (dated 16 July 2002) to inform us that all recommendations had been adequately addressed.

There are many institutions in Australia associated with the above national study and we have submitted the minor amendments requested by the HSRRB to the IRB's at each collaborating site. In addition, a number of the institutions do not have a Federalwide Assurance and so we have had to work with these hospitals to complete applications for a Single Project Assurance. We are currently awaiting documentation of the final IRB approval and, in some cases, SPA applications, from all of these institutions. Once this has been received by the HSRRB, the project can receive final approval from the Department of Defense.

Tasks Outlined in the Approved Statement of Work
Core A: Epidemiology

Task 1- Preliminary Work (Prior to start date)

(a) Data-collection instruments will be finalised and piloted based on practical experience in a previous study (Survey of Women's Health)

The **main study questionnaire** has been through an extensive development phase to ensure measurement of exposure to a comprehensive range of established and putative causal factors, potential confounders and novel factors

of interest. Because the instrument was to be used by cases and disease-free controls from a range of educational backgrounds, we invested much effort in producing a document that was clear, simple and unambiguous. When the final content of the questionnaire was established, we focused on the style and formatting to ensure readability, clarity and consistency, and to ensure that items were relevant to people with and without disease. We then undertook pilot testing on a convenience sample of neighborhood controls from a range of educational and occupational backgrounds (specifically non-medical, non-research) to identify inappropriate use of jargon and potential sources of ambiguity or irrelevance. (Each pilot respondent was invited to provide direct feedback, and they did). The revised questionnaire was then tested in two groups of patients for whom clinical records were available to permit validation of responses. The returned questionnaires showed a high degree of completeness and the responses demonstrated a high level of internal and external validity.

The **food-frequency questionnaire** has been updated to reflect changing dietary practices since the original instrument was developed more than 10 years ago. We have also added additional items to address specific hypotheses concerning the role of soy products and xeno- and phytoestrogens in ovarian carcinogenesis. This questionnaire has been trialed in a group of cancer-free patients and minor modifications made to improve readability.

Case-recruitment: More extensive pilot testing of the study instruments and recruitment protocols has been conducted in Queensland using limited local funds. Women diagnosed with ovarian cancer at the Royal Women's Hospital, Brisbane (since 1 January, 2002) and at the Mater and Wesley Hospitals (since 1 July 2002) have been recruited and asked to complete the main study questionnaire. To date 102 cases have been identified of which 92 were potentially eligible and 82 (90% of eligible cases) have consented to participate. Tissue collection started in April 2002 and blood collection began in July and 34 tissue and 10 blood samples have been collected to date. We are in the process of cross-checking with the local Gynaecology-Oncology register and the State Cancer Registry to identify any cases we have missed and will then modify our ascertainment procedures if necessary to improve capture in the future.

Control recruitment: we are currently trialing the control recruitment methods in a separate study of oesophageal cancer. In response to the first batch of 49 study invitations sent, 32 men and women (65%) have agreed to participate to date, and a few people are still to be contacted. Participation rates are slightly higher among women than men thus we anticipate that we will be able to achieve the estimated participation rate of 70% for AOCS.

(b) Institutional Review Board approval will be obtained from all participating hospitals and institutions (to be prior to start date)

Institutional Review Board approval has been obtained from the collaborating sites (except Royal Women's Hospital, Victoria, which is in progress). As mentioned above, we have submitted the revised study documents with minor amendments (incorporating the changes requested by the DoD HSRRB) to all the collaborating sites and are waiting approval for these. We will commence

recruitment for AOCS as soon as we receive final approval from the DoD and we expect this to be within the next few weeks.

(c) **Identification of project manager, data manager and nurse-interviewers to start on Day 1.**

A Project Manager (Sue Moore) has been recruited to set up and manage the Epidemiology Core.

A centralized Data Processing Unit within the Cancer Epidemiology Unit at the Queensland Institute of Medical Research has been established and a Data Manager (Karen Harrap) has been recruited to develop and maintain the master databases for the Epidemiology Core.

Several nurse interviewers have been identified and interviewed and will commence once final approval is received from DoD.

Task 2- Set-up (months 1-2)

(a) **Finalise details of case identification system in each of the major centres (month 1)**

The Epidemiology Project Manager (together with the Biospecimen Project Manager) has been working with clinicians, pathologists and nursing staff at each of the major centres to establish a system for case ascertainment and recruitment appropriate for each site. Processes for data and sample collection are also in place at each of the major centres. Once final approval is obtained, the research nurses will maintain regular contact with the gynecology-oncology clinics and wards to identify eligible women as soon as possible after diagnosis.

We have participated in the Australian New Zealand Gynaecological Oncology Group (ANZGOG) meetings in 2001 and 2002, presenting details of the study on both occasions. ANZGOG is affiliated with the US GOG. There is comprehensive representation at the meetings by pathologists and gynaecological oncologists at the meetings and they have provided an excellent opportunity to provide information to key participants. There is enthusiastic endorsement of the study by ANZGOG members.

(b) **Training of interviewers in Brisbane (month 1)**

Once final approval is received, the Nurse Interviewers will be trained on-site by the Epidemiology and Biospecimen Project Managers and the specific recruitment details for each site will be finalised. We plan to bring all of the nurses to Brisbane in November for more detailed training and to trouble-shoot any problems that have arisen in the early stages of recruitment.

(c) **Development of computer data-bases (Access) for data-entry**

Recruitment "Tracking" Database

We have developed a secure internet-based database for the state-based study nurses to register newly identified cases and to allow us to track all stages of recruitment, consent and data and sample collection. The database is constructed using the MySQL Relational Database Management System and comprises 31 tables. A dedicated web server that sits behind the QIMR firewall provides the platform, with web services effected via the Apache web server that employs the Secure Socket Layer to provide an encrypted channel for all communication

between client and server machines. The database has been pilot tested and is ready to come on line as soon as recruitment begins. The development of other linked databases for entry of questionnaire data is underway.

Tasks 3, 4 and 5 cover recruitment of cases and controls and data entry. These will be initiated once final approval for the study is received.

Task 3- Recruitment of cases (n>1000). (Ongoing months 2-36)

(a) Cases will be identified by the nurse-interviewers on an ongoing basis through participating hospitals and clinics with additional checks run through the state cancer registries
(b) Treating physicians will be contacted to obtain permission to contact the case
(c) Cases will be contacted and interviewed and biological samples collected
(d) Tumor blocks and copies of pathology records will be obtained

Task 4- Recruitment of population controls (n>1000). (Ongoing months 2-36)

(a) Potential controls will be selected at random through the Commonwealth electoral roll on a weekly basis and frequency matched by age and geographic region to the distribution of cases identified the previous week
(b) Invitation letters will be sent to controls
(c) Telephone follow-up of controls
(d) Interview of controls and collection of blood and urine samples

Task 5- Data entry / checking / cleaning. (ongoing months 3-42)

(a) Data will be entered into the databases on an ongoing basis
(b) Data will be cleaned using frequency and range checks, implausible values will be cross-checked against the original questionnaires and corrected if necessary

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<u>Core B- Biospecimens</u>
Task 1- Preliminary Work (Prior to start date)

a) Institutional Review Board approval will be obtained from all participating hospitals and institutions (to be prior to start date)

Institutional Review Board approval has been obtained from the collaborating sites (except Royal Women's Hospital, Victoria, which is in progress). As mentioned above, we have submitted the revised study documents with minor amendments (incorporating the changes requested by the DoD HSRRB) to all the collaborating sites and are waiting approval for these. We will commence recruitment for AOCS as soon as we receive final approval from the DoD and we expect this to be within the next few weeks.

b) Recruitment of data manager and specimen processing staff

A Project Manager (Nadia Traficante) has been recruited to coordinate the biospecimen core and oversee management of specimen processing staff. To date, this has involved establishing biospecimen collection and processing protocols, ordering consumables and liasing with clinicians, pathologists and ward staff to establish site-specific sample collection procedures.
A Data Manager (Sian Fereday) has been recruited to develop the biospecimen core databases and manage data entry staff.
Specimen processing staff have also been identified and interviewed and will commence once final approval has been received.

c) Further refinement of computer data-bases (Access) for data-entry

Further refinement of the biospecimen database to ensure the appropriate tracking from collection through processing to final usage of all specimens has been completed. In addition, the database also allows all biospecimen data to be linked to diagnostic pathology data and central pathology review information.

Task 2- Set-up (months 1-2)

a) Finalise details of case ascertainment system in each of the major centres (month 1)

The Project Managers have been working with clinicians, pathologists and nursing staff at each of the major centres and established a system for case ascertainment and recruitment at each of the collaborating sites. Processes for data and sample collection are also in place at each of the major centres. Once final approval is obtained, the research nurses will maintain regular contact with the gynecology-oncology clinics and wards to identify eligible women as soon as possible after diagnosis.

b) Obtain minor equipment and consumables

We have purchased a Liquid Nitrogen tank for storage of blood, tissue and urine samples. Minor equipment (gilsons, pipettes) and consumables (including blood, tissue and urine collection tubes, syringes, needles, scalpel blades, forceps, reagents for specimen processing, Guthrie cards, gloves, tips, bio-bottles and consignment notes) for the biospecimen core have been purchased.

Tasks 3, 4 and 5 below cover ascertainment of samples, sample processing and dispatch and data entry. These will be initiated once final approval for the study is received.

Task 3- Ascertainment of samples. (Ongoing months 2-36)

a) Nurse-interviewers to liase with Biorepository head, notifying of incoming shipment of samples

b) **Nurse-interviewers to provide Biorepository staff with details of pathology blocks from cases to be requested. Biorepository staff to coordinate temporary block acquisition**

c) **Blood and urine samples shipped overnight from national sites. Fresh frozen samples from interstate stored at at centres at –80°C, shipped on dry ice at monthly intervals. Blocks from pathology clinics requested on a monthly basis.**

Task 4. Sample processing and dispatch. (Ongoing months 2-36)

a) **Incoming samples of blood, urine, fresh frozen tissue and blocks to be processed as described in methods**

b) **Requested samples shipped to centres in general on a monthly basis but immediately available if needed**

c) **Sample backup to QIMR sent as batches on a monthly basis**

d) **Periodic quality control procedures to validate sample integrity**

Task 5. Data entry / checking / cleaning. (ongoing months 3-42)

a) **Data will be entered into the databases on an ongoing basis**

b) **Provide data for analysis as required**

Project 1: Molecular Subtype Analysis of Ovarian Cancer

Task 1 **Initial DNA microarray analysis with ~300 archival fresh frozen samples (months 1-12)**

Initial microarray analysis will begin once final approval for the study is received.

Tasks 2, 3 and 4 listed below cover Years 2, 3 and 4 and do not apply to this annual report.

Task 2 **Progressively switich to microarray analysis of prospectively collected samples (months 12-42)**

Task 3 **Ongoing statistical analysis of expression results (months 3-42)**

Task 4 **Full statistical analysis of expression data and preparation of manuscripts (months 42-48)**

Project 2: Determinants of Epithelial Ovarian Cancer- by histologic subtype and tumor behaviour

This project will not formally commence until epidemiologic data collection is complete and it will run through the 4^{tth} year of the program. During Year 4 analysis of the specific hypotheses will proceed in parallel under the guidance of the PI and Co-investigators.

Project 3: Low-risk genes for epithelial ovarian cancer

Task 1 **To establish the 16 single nucleotide polymorphism (SNP) genotyping assays, including identification of genotyping controls (months 1-18)**

Task 2 **To genotype the cases from the Survey of Women's Health Study and controls from the Australian Breast Cancer Family Study for 16 SNPS (months 6-24)**

Genotyping assays have been established for 7 SNP's as indicated in the table below. Genotyping for another system (HSD17b1: Ser313Gly) is nearly complete. Genotyping assay design is currently under way for a further 2 systems (HSD17b4: Trp511Arg and HSD17b1: A-27C).

Gene (Symbol)	Polymorphism	Design
Androgen Receptor (AR)	CAG_n	Completed
Progesterone Receptor (PR)	C44T	Not Commenced
Progesterone Receptor (PR)	G332A	Not Commenced
Aromatase (CYP19)	C>T 3'UTR	Completed
5alpha-reductase (SRD5A2)	Val89Leu	Completed
17beta-hydroxysteroid dehydrogenase (HSD17b1)	A-27C	Commenced
17beta-hydroxysteroid dehydrogenase (HSD17b1)	Ala238Val	Completed
17beta-hydroxysteroid dehydrogenase (HSD17b1)	Ser313Gly	Completed
17beta-hydroxysteroid dehydrogenase (HSD17b4)	Trp511Arg	Commenced
BRCA2	Asn372His	Completed
X-ray cross complementation (XRCC2)	Arg188His	Completed
X-ray cross complementation (XRCC3)	Thr241Met	Completed
X-ray cross complementation (XRCC3)	CA_n	Not Commenced
RAD50	Arg884His	Not Commenced
RAD52	Ser347Ter	Not Commenced
RAD52	Tyr418Ter	Not Commenced

Tasks 3, 4, 5 and 6 listed below cover Years 3 and 4 and do not apply to this annual report.

Task 3 **To genotype the cases and controls from the Australian Ovarian Cancer Study for 16 SNPs (months 25-42)**

Task 4 **To perform genotyping for 2 short tandem repeat (STR) polymorphisms on both case-control studies (months 36-42)**

Task 5	**Statistical analysis of the genotyping results from the Survey of Women's Health Study and controls from the Australian Breast Cancer Family Study (months 24-36)**
Task 6	**Full statistical analysis of the genotyping results (months 40-48) and preparation of manuscripts**

KEY RESEARCH ACCOMPLISHMENTS

N/A

REPORTABLE OUTCOMES

N/A

CONCLUSIONS

Funding for the study began in September 2001 and we have since obtained ethics approval from the collaborating sites associated with the study (except Royal Women's Hospital, Victoria, which is in progress). We have submitted minor amendments (incorporating the changes requested by the DoD HSRRB) to all the collaborating sites and are waiting approval for these. We will commence recruitment for AOCS as soon as we receive final approval from the DoD and we expect this to be within the next few weeks.

We have been unable to start recruitment for the study as final approval from the DoD has not yet been received. However, the Project Managers have been working with clinicians, pathologists and nursing staff at each of the major centres and established a system for case ascertainment and recruitment at each of the collaborating sites. Processes for data and sample collection are also in place at each of the major centres and all minor equipment and consumables have been ordered.

A pilot study in Queensland (using local pilot funding) has tested the recruitment and data/sample collection procedures and the main study questionnaire. The control recruitment procedures have been developed and tested in a separate study of oesophageal cancer. The tracking database for the study has also been developed and piloted and minor technical problems have been resolved.

We are mindful of the fact that DoD funds cannot be used for preparatory work associated with the study, other than that outlined above, until final approval is met. Funding accrued during this period that has not been used for accepted activities has been reserved for use once the study commences.

REFERENCES
N/A

APPENDICES
N/A